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REVIEW

Diagnostic and prognostic potential of circulating miRNAs for intracranial aneurysms

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Abstract

Intracranial aneurysm (IA) is an abnormal focal dilation of an artery in the brain that results from a weakening of the inner muscular layer of a blood vessel wall. IAs represent the most common etiology of nontraumatic subarachnoid hemorrhage (SAH). Despite technological advances in the treatment and use of new diagnostic methods for IAs, they continue to pose a significant risk of mortality and disability. Thus, early recognition of IA with a high risk of rupture is crucial for the stratification of patients with such a formidable disease. MicroRNAs (miRNA) are endogenous noncoding RNAs of 18–22 nucleotides that regulate gene expression at the post-transcriptional level through interaction with 3′-untranslated regions (3′UTRs) of the target mRNAs. MiRNAs are involved in the pathogenesis of IAs, including in the mechanisms of formation, growth, and rupture. It is known that in many biological fluids of the human body, such as blood or cerebrospinal fluid (CSF), numerous miRNAs, called circulating miRNAs, have been detected. The expression profile of circulating miRNAs represents a certain part of the cells in which they are modified and secreted in accordance with the physiological or pathological conditions of these cells. Circulating miRNAs can be secreted from cells into human biological fluids in extracellular vesicles or can be bound to Ago2 protein, which makes them resistant to the effects of RNAse. Therefore, circulating miRNAs are considered as new potential biomarkers of interest in many diseases, including IA.

Keywords Circulating miRNAs · Biomarkers · Intracranial aneurysms · aSAH

Introduction

The prevalence of intracranial aneurysms (IAs) in the general population is estimated to be 2–5% [1]. IAs are most often found in places of separation of arteries, where high shear stress on the vascular wall affects. Histologically, IAs have lost the inner elastic plate of the vessel wall, which separates the intima from the concentric smooth muscle layer called the media. Therefore, the loss of elastic fibers contributes to the protrusion of the remaining layers of the vessel [2]. Most IAs remain asymptomatic throughout the patient's life. However, rupture of the aneurysm and subsequent subarachnoid hemorrhage (SAH) is associated with 70% mortality and 30–50% of survivors remain disabled [3]. The diagnosis of IAs is currently based on imaging techniques such as computed tomography angiography (CTA), magnetic resonance angiography (MRA), and selective cerebral angiography (SCA). In contrast to tumors, which have many specific and non-specific plasma or serum markers that can be used both for diagnosis, prognosis, and monitoring of therapy, there are no established non-invasive biomarkers for patients with IAs [4]. Patients with a family history of cases of SAH associated with rupture of an aneurysm (aSAH) (two or more first-degree relatives of kinship) have a significant risk of detecting IAs and the risk of aSAH [5, 6]. A number of studies have shown that early detection with MRA and treatment of revealed IAs in such

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people reduced the risk of rupture of aneurysms [5–7]. Other studied groups of people in these studies included patients with one first-degree relative with aSAH, patients who suffered an episode of aSAH, and people with modified risk factors. In these patient populations, screening with MRA is less or less cost-effective. The inefficiency of screening using MRA to identify all individuals with a high risk of rupture of aneurysms underscores the need for new screening methods to detect IAs or IAs with a high risk of rupture. Taking into account that CTA/MRA and /SCA are either unavailable or do not give obvious signs of a possible rupture of IAs, accurate and reliable analysis of the profile of circulating miRNAs in biological fluids can help in the early diagnosis and prediction of rupture, as well as probable mortality and morbidity or prognostic outcome of patients with aSAH in order to stratify patients entering medical institutions.

MicroRNAs (miRNAs) are a class of small, endogenous RNAs of 18–22 nucleotides in length that inhibit gene expression at the post-transcriptional level by binding to the 3′-untranslated region (3′-UTR) of mRNAs-targets (Fig. 1) [8]. MiRNAs are involved in almost all biological processes, including cell proliferation, apoptosis, and cell differentiation [8, 9]. There are a number of studies that have shown a direct relationship between miRNAs and the formation and development of IAs (Table 1) [10–20]. Most miRNAs are expressed within the cells themselves. However, in many biological fluids of the human body, such as blood and cerebrospinal fluid (CSF), numerous miRNAs, called circulating miRNAs, have been found [21]. To date, there are three known ways of secretion of circulating miRNAs: (1) passive secretion from damaged cells due to apoptosis or necrosis; (2) active secretion using extracellular vesicles (EV); (3) active secretion using an RNA-binding protein-dependent pathway [22] (Fig. 1). Such miRNAs are resistant to nucleases, which makes them attractive as potential biomarkers. The expression profile of circulating miRNAs varies significantly with various human diseases, including cardiovascular diseases [23]. The detection of changes in the expression of circulating miRNAs in IAs suggests the creation of new diagnostic and prognostic markers. Meanwhile, the analysis of miRNA target genes could open new horizons in understanding the pathogenesis and treatment of IAs. In this study, we review current knowledge regarding circulating miRNAs for IAs and aSAH, with the aim of better understanding the functions of miRNAs in this pathology and the potential introduction of circulating miRNAs as new biomarkers in clinical practice.

Literature research strategy

We performed a comprehensive search for original papers demonstrating the diagnostic and prognostic power of circulating miRNAs in IAs. Databases including PubMed, Embase, the Cochrane Library Database, and Google Scholar were used to obtain all relevant studies up to September 2020. Key words including “intracranial aneurysm” or “subarachnoid hemorrhage” or “vasospasm” or “rupture” or “pathogenesis”, “microRNA” or “miRNA” or “miR” or “non-coding RNA” or “exosome” and “plasma” or “serum” or “circulating” or “blood” or “biomarker”. In addition, a reference list of each relevant study was manually searched to obtain other valuable articles. The flow chart of the systematic review is shown in Fig. 2.

Circulating miRNAs as biomarkers

To date, several biomarkers for IAs have been investigated. These include various immunological, genetic, inflammatory biomarkers, as well as factors associated with angiogenesis or cell adhesion [24]. No biomarker was found that could independently predict the formation, progression, and rupture of IAs. Circulating miRNAs as non-invasive biomarkers of IAs are less studied (Table 2), although they may be more sensitive and accurate in the diagnosis and prognosis of unruptured IAs and ruptured IAs: (1) non-invasive detection technique; (2) circulating miRNAs are very stable and have a long half-life in the sample; (3) the profile of circulating miRNAs may exhibit high specificity depending on tissue and disease; (4) a change in the profile of certain circulating miRNAs can detect IAs associated with a high risk of rupture; (5) circulating miRNAs as a promising tool for faster and more accurate diagnosis of stroke subtypes (aSAH from spontaneous SAH); (6) can be detected in the early stages of the development of the disease, whereas protein biomarkers are detected in biofluids only when a significant part of the damage has already occurred; and (7) due to their small size and chemical composition, circulating miRNAs are less complex molecules than most biological molecules in the blood or CSF, which simplifies the analysis [21–23, 48].

Circulating miRNAs for unruptured and ruptured IAs

Sheng et al. demonstrated that a high level of expression of circulating miR-1297 in the serum of patients with aSAH was associated with a poor prognosis [25]. The study involved a total of 552 serum samples: 128 aSAH patients and 40 healthy controls. In their study, they found a change in serum miR-1297 expression at the four time points (24, 72, and 168 h; and 14 days). Higher expression levels of serum miR-1297 were demonstrated after 24 and 72 h. The areas under ROC curve (AUCs) of miR-1297 at the four time points for distinguishing the aSAH patients from healthy controls were 0.80, 0.94, 0.77, and 0.59, respectively. These findings suggest that serum miR-1297 had a high power to distinguish aSAH patients from healthy controls, especially at 72 h after aSAH. In

Table 1 MiRNAs involved in intracranial aneurysms (IAs)

miRNA	Gene-target	Sample studied	Cellular origin	Regulation	Related functions	Type assay	Ref.
miR-370-3p	AKT/FOXO1	HUASMCs, human cerebral arteries tissue	VSMCs	Up	Inhibits VSMCs proliferation	qRT-PCR	[10]
18 miRNAs and 11 miRNAs are clusters	CCL2, MMP14, IL18, TLR4, KLF4, BCL2L1, CSF1 etc.	Human cerebral arteries tissue	ECs, VSMCs, immune cells	Down	Migration of phagocytes; proliferation of mononuclear leukocytes, VSMCs, and ECs; cell movement of mononuclear leukocytes, cell movement of VSMCs; apoptosis of ECs etc.	qRT-PCR	[11]
miR-23b-3p	PTEN	Human cerebral and pulmonary arteries tissue	VSMCs	Down	Affected the viability and apoptosis of VSMCs.	qRT-PCR	[12]
miR-29a	caspase-3, -8, -9, cytochrome c and Mcl-1	HBVSMCs, 293-T cells, mouse cerebral arteries tissue, human blood	VSMCs	Up	Regulate the mitochondrial apoptosis pathway	qRT-PCR, microarray	[13]
miR-448-3p	KLF5, MMP2, and MMP9	Rat cerebral arteries tissue	VSMCs	Down	Anti-inflammatory effect	qRT-PCR	[14]
miR-31a-5p	Axin1-mediated Wnt/β-catenin pathway	Rat aorta tissue	EPCs	Up	Important regulator of EPCs mobilization and endothelialization	qRT-PCR	[15]
miR-143/145	KLF5	HASMCs, human blood	VSMCs	Down	VSMC phenotypic modulation	qRT-PCR	[16]
miR-125b	NOS1	Human cerebral and superficial temporal arteries tissue	VSMCs	Down	Modulating proliferation and apoptosis of VSMCs	Microarray	[17]
miR-9	MYOCD	Human cerebral arteries tissue	VSMCs	Up	Negatively affects with the viability and contractility of VSMCs.	qRT-PCR	[18]
miR-21	PAIP2B	Human cerebral arteries tissue	Cerebral vascular wall	Up	Collagen formation, inflammation regulation, lipid metabolism, smooth muscle phenotypic modification, and extracellular matrix remodeling	Deep RNA sequencing	[19]
miR-143	COL1A1, COL5A1, COL5A2, MARCKS and TANC2			Down			
miR-145	ABCA1, ADAMTS2, and BCAT1			Down			
miR-155	MMP-2	Human cerebral arteries tissue, HPASMCs, A7R5C, human blood	VSMCs	Up	VSMCs dysfunction and ECM disruption	qRT-PCR	[20]

miR, MicroRNA; *AKT*, RAC-alpha serine/threonine-protein kinase; *FOXO1*, Forkhead box protein O1; *CCL2*, C-C Motif chemokine ligand 2; *MMP*, Matrix metalloproteinase; *IL18*, Interleukin 18; *TLR4*, Toll-like Receptor 4; *KLF*, Krueppel-like factor; *BCL2L1*, B cell lymphoma like 1; *CSF1*, Macrophage colony-stimulating factor 1; *PTEN*, Phosphatase and tensin homolog deleted on chromosome 10; *Mcl*-1, Myeloid cell leukemia sequence-1; *NOS1*, Nitric oxide synthase 1; *MYOCD*, Myocardin; *HUASMCs*, Human umbilical artery smooth muscle cells; *HBVSMCs*, Human brain vascular smooth muscle cells; *VSMCs*, Vascular smooth muscle cells; *EPCs*, Endothelial progenitor cells; *ECs*, Endothelial cells; *HASMCs*, Human aortic smooth muscle cells; *qRT-PCR*, Real-time reverse transcription-polymerase chain reaction; *PAIP2B*, Poly(A)-binding protein interacting protein 2B; *COL1A1*, Collagen, type 1, alpha 1; *COL5A1*, Collagen, type 5, alpha 1; COL5A2, Collagen, type 5, alpha 2; *ABCA1*, cholesterol efflux regulatory protein; *ADAMTS2*, A disintegrin and metalloproteinase with thrombospondin motifs 2; *BCAT1*, branched chain amino acid transaminase 1; *ECM*, Extracellular matrix; *HPASMCs*, human pulmonary artery smooth muscle cells

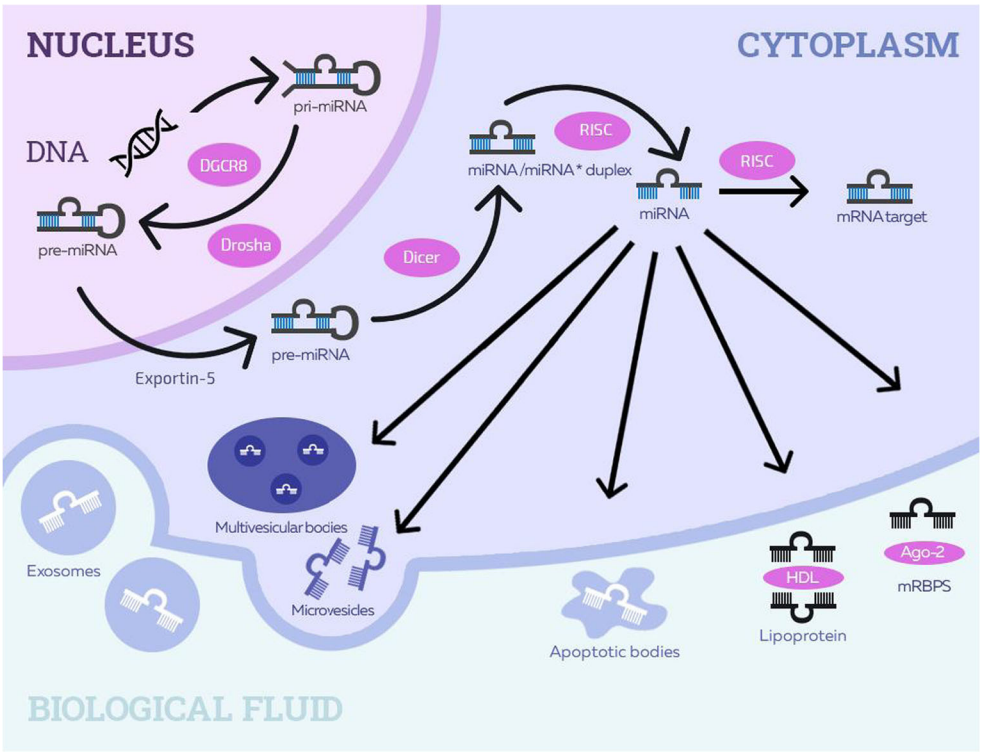

Fig. 1 The primary miRNA transcript (pri-miRNA) is first transcribed in the nucleus by RNA polymerase II, which is subsequently cleaved by Drosha/DGCR8 proteins into a miRNA precursor (pre-miRNA). Pre-miRNA is exported from the nucleus to the cytoplasm of the cell using the Exportin-5 protein and loaded onto Dicer; then the loop of pre-miRNA is cleaved, forming a double-stranded structure consisting of mature miRNA and antisense miRNA (miRNA duplex). The latter usually degrades, while the master strand of the mature miRNA is incorporated into the miRNA-induced silencing complex (RISC), which leads to the suppression of gene expression by binding to the 3′-untranslated region (3'-UTR) of specific mRNA targets. In turn, miRNAs can be exported from cells into biological fluids as part of extracellular vesicles (active secretion through exosomes and microvesicles) and be associated with RNA-binding proteins (mRBPs), in particular with proteins Argonaute 2 (miRNA-Ago2 complex). MiRNAs can also be found in apoptotic bodies and are associated with high-density lipoproteins (HDL)

addition, in aSAH patients, serum levels of miR-1297 at these four time points negatively correlated with aSAH severity as scored by WFNS grade. Sheng et al. also tried to check the change in the expression of the circulating miR-502-5p in the serum of aSAH ($n = 129$) patients at the four time points (24, 72, and 168 h; and 14 days) [26]. They demonstrated that the serum levels of miR-502-5p in aSAH patients increased at first 24 h post-aSAH when compared with healthy controls ($n = 40$), and peaked at 168 h or 7 days. Notably, from day 7 to day 14, expression levels of circulating miR-502-5p decreased. In another study, expression levels of circulating miR-502-5p, miR-4320, and miR-1297 in the serum were markedly increased in the aSAH patients ($n = 63$) compared with the controls ($n = 13$) with the AUC value of 0.958, 0.843, and 0.950 respectively [27]. They also found that the expression of the serum levels of miR-502-5p and miR-1297 in aSAH patients with higher WFNS grade was higher than that with lower WFNS grade. The authors developed and validated a novel diagnostic and prognostic tool based on serum miR-502-5p and miR-1297 for aSAH.

The mir-143/145 cluster is fairly extensively studied in vascular biology and the pathophysiology of cardiovascular disease. And the underpinning biogenesis and processing of the miR-143/145 cluster has been extensively studied by different research groups [49, 50]. Feng et al. analyzed the relationship between plasma miR-143/145 and serum matrix metalloproteinase-9 (MMP-9) levels in 24 patients with unruptured or ruptured IAs [28]. Serum and plasma were isolated femoral artery blood after they were punctured for DSA. It has been shown that plasma levels of miR-143/145 were significantly lower in patients with IAs than in healthy controls. Serum levels of MMP-9 were significantly higher in patients with ruptured IAs than in patients with unruptured IAs and healthy controls. However, plasma levels of miR-143/145 were not significantly correlated with serum MMP-9 levels. Lower plasma levels of the miR-143/145 cluster may be associated with IA formation and higher serum levels of MMP-9 may be associated with IA rupture. In addition, Xu et al. confirmed that the serum levels of the miR-143/145 cluster were significantly decreased in IAs patients compared to that of healthy controls, indicating that the miR-143/145 cluster could be involved in the formation and progression of IAs [16].

MiR-155 has been reported to participate in the process of vascular remodeling, which is critical for complex adaptive

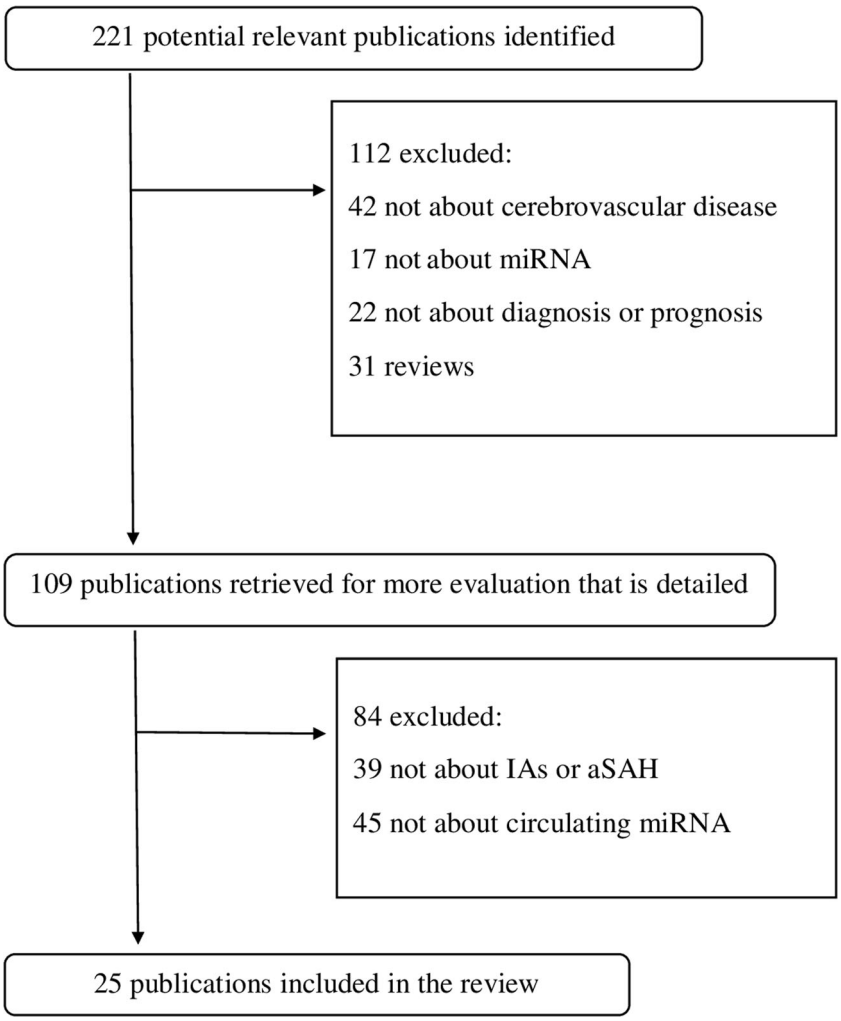

Fig. 2 The flow chart of study section

reactions in cardiovascular diseases, including IAs [20, 51]. For example, Yang et al. found the relative expression of miR-155 was upregulated in the tissue specimens of unruptured IA patients compared with hat in the tissue specimens of ruptured IA patients [20]. Besides the authors identified matrix metalloproteinase-2 (MMP-2) as a potential target of miR-155, with a possible binding site located in the 3′-UTR of MMP-2. In addition, the results showed that the relative expression of serum miR-155 was upregulated in the unruptured IAs group patients ($n = 46$) compared with that in the ruptured IAs group patients ($n = 48$). This evidence suggests that a change in the expression of serum miR-155 may serve to predict a rupture IA. Wang et al. reported a close association between increased expression of circulating miR-29a in plasma and rupture IA, Hunt-Hess level, and surgical timing [29]. Furthermore, patients with low miR-29a expression had longer disease-free survival and overall survival than those with high miR-29a expression. Using the real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR), Meeuwsen et al. identified the three specific circulating miRNAs, miR-183-5p, miR-200a-3p, and miR-let-7b, that can discriminate between IAs patients ($n = 11$) and healthy controls ($n = 15$) [30]. However, circulating miR-200a-3p level was increased only in aSAH patients ($n = 15$), and not in IA patients, which might suggest that miR-200a-3p influences the risk of rupture IA. Maybe these circulating miRNAs may be used biomarkers for identifying individuals at high risk of IA development and its subsequent rupture. In plasma samples of 40 IA patients (20 unruptured and 20 ruptured), a microarray study identified 20 circulating miRNAs that were unanimously changed in both ruptured and unruptured patients [31]. The authors further performed quantitative real-time polymerase chain reactions for selected target microRNAs. Among PCR-detectable circulating miRNAs, miR-16 and miR-25 levels were significantly higher in IA patients. Both circulating miR-16 and miR-25 were good predictors for IA, with AUC being 0.857 and 0.852 respectively. These results suggest that circulating miR-16 and miR-25 may be used as novel biomarkers in assessing the likelihood of occurrence of IAs in high-risk individuals.

Supriya et al. demonstrated that the expression of plasma (peripheral venous blood) miRNAs was significantly altered

Table 2 Circuiting miRNAs as diagnostic, prognostic, or predictive biomarkers in unruptured intracranial aneurysms (IAs) and ruptured IAs

miRNA	Sample	Regulation	Diagnostic of IAs or aSAH	Prognostic of aSAH	Predictive of rupture IAs	Sensitivity %	Specificity %	AUC	Ref.
miR-1297	Serum	Up	Yes	Yes	No	73	86	0.94	[25]
miR-502-5p	Serum	Down	Yes	Yes	No	95.3	92.5	0.970	[26]
miR-502-5p	Serum	Up	Yes	Yes	No	88.2	99.2	0.958	[27]
miR-4320		Up	Yes	No	No	73.7	91.2	0.843	
miR-1297		Up	Yes	Yes	No	87	98.8	0.950	
mir-143	Plasma	Down	Yes	No	Yes	/	/	/	[28]
mir-145		Down	Yes	No	Yes	/	/	/	
mir-143	Serum	Down	Yes	No	Yes	/	/	/	[16]
mir-145		Down	Yes	No	Yes	/	/	/	
miR-155	Serum	Up	Yes	No	Yes	/	/	/	[20]
miR-29a	Plasma	Down	Yes	Yes	Yes	80.6	94.1	0.992	[29]
miR-183-5p	Plasma	Down	Yes	No	Yes	69	98	0.83	[30]
miR-200a-3p		Up	Yes	Yes	No	55	94	0.74	
miR-let-7b		Down	Yes	No	Yes	81	100	0.92	
miR-16	Plasma	Up	Yes	No	Yes	/	/	0.857	[31]
miR-25		Up	Yes	No	Yes	/	/	0.852	
miR-15a-5p, miR-34a-5p, and miR-374a-5p	Plasma	Up	Yes	No	No	/	/	Comb. 0.865	[32]
miR-146a-5p, miR-376c-3p, miR-18b-5p, miR-24-3p, and miR-27b-3p		Down	Yes	Yes (only for miR-146a-5p and miR-27b-3p)	No	/	/	Comb. 0.998	
90 miRNAs	Venous whole blood	Up	/	/	/	/	/	/	[33]
106 mature miRNAs and 90 miRNA precursors	Venous whole blood	Up	/	/	/	/	/	/	[34]
miR-126	Serum	Up	Yes	No	Yes	/	/	0.966	[35]
miR-92a and let-7b	CSF	Down	No	Yes	No	/	/	/	[36]
miR-491		Up							
miR-3177-3p	Venous whole blood	Up	No	Yes	No	/	/	/	[37]
miR-132-3p	Plasma	Up	Yes	Yes	Yes	/	/	0.97	[38]
miR-324-3p		Up						0.75	
miR-15a	Plasma and CSF	Up	No	Yes	No	/	/	/	[39]
	Venous whole blood	Up	Yes	Yes	No	/	/		[40]

Table 2 (continued)

miRNA	Sample	Regulation	Diagnostic of IAs or aSAH	Prognostic of aSAH	Predictive of rupture IAs	Sensitivity %	Specificity %	AUC	Ref.
miR-3195, miR-4788, and miR-1914								Comb. 0.976 and comb. 0.902	
miR-486-5p	Venous whole blood	Down	No	Yes	No	/	/	/	[41]
146a-5p, miR-21 and miR-221	CSF	Up	No	Yes	No	/	/	/	[42]
miR-451a	CSF	Up	No	Yes	No	/	/	/	[43]
Exosomal miR-145-5p Exosomal miR-29a-3p	Plasma	Up Up	Yes	No	Yes	/	/	0.737 0.791	[44]
Exosomal miR-630	CSF	Down	No	No	Yes	/	/	/	[45]
Exosomal 20 Exosomal miRNAs Exosomal 5 miRNAs	Plasma	Up Down	Yes	No	No	/	/	0.895	[46]
Exosomal (miR-369-3p miR-136-3p, and miR-410-3p) Exosomal (miR-195-5p, miR-486-3p, and miR-193b-3p)	Plasma	Down Up	No	Yes	No	/	/	/	[47]

AUC, area under ROC curve; AUC ≥ 0, 70 is considered diagnostically significant for the biomarker; /, not mentioned in the article; comb., combined; *miR*, microRNA; *CSF*, Cerebral spinal fluid

in patients with aSAH ($n = 20$) and identified 8 circulating miRNAs that could serve as candidate biomarkers for IA rupture [32]. Study showed that 3 upregulated miRNAs (miR-15a-5p, miR-34a-5p, and miR-374a-5p) and 5 downregulated miRNAs (miR-146a-5p, miR-376c-3p, miR-18b-5p, miR-24-3p, and miR-27b-3p) with high predicted probability. Further, the expression levels of the 8 candidate circulating miRNAs were significantly dysregulated only in aSAH cases and not in patients with SAH due to other causes ($n = 25$) and control group ($n = 20$). Herewith circulating miR-146a-5p and miR-27b-3p were associated with clinical outcomes in patients with aSAH. In addition, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that these miRNAs interact with 2896 genes, and pathway enrichment analysis of target genes showed that these were involved in transforming growth factor-β, mitogen-activated protein kinase, focal adhesion, and the phosphoinositide 3 kinase/protein kinase B signaling pathway. Thus, the authors hypothesize that the identified differentially expressed candidate miRNAs may play pivotal roles in IAs pathology by regulating many signaling pathways related to inflammation.

Morga used next-generation sequencing (NGS) to analyze the expression of multiple classes of small RNAs (sRNAs) in venous whole blood of patients groups in the acute phase of IA rupture (first 72 h) ($n = 19$), in the chronic phase (3–15 months) ($n = 20$), and controls ($n = 20$) [33]. They identified 542 differentially expressed sRNAs, where 90 miRNAs, among the studied groups with notable differences in upregulated and downregulated sRNAs between the groups and sRNAs categories. MiRNAs showed a substantial increase in RNA abundance that was sustained after IA rupture particularly the chronic phase after IA rupture. If the results are further validated, there is a translational clinical potential in the presented observations. Namely, levels of sequences of miRNAs could serve as potential IA biomarkers; however, further studies are needed. Subsequent studies further demonstrated change expression of circulating miRNA using NGS in patients in the acute phase of IA rupture (first 72 h) ($n = 19$), in the chronic phase (3–15 months) ($n = 20$), and in controls ($n = 20$) [34]. The authors found that 106 circulating mature miRNAs and 90 miRNA precursors were differentially expressed in venous whole blood among the groups. The regulated miRNAs were involved in a variety of pathways, and the top pathway involved cytokine-cytokine receptor interactions. By using different tools, including WikiPathways, GO, and Human MicroRNA Disease Database (HMDD), they were identified that these miRNAs targeted the inflammatory factors high mobility group box 1 protein (HMGB1) and fas ligand gene (FASLG). These results may aid in the elucidation of the mechanisms that orchestrate the inflammatory response to IA rupture and studying these circulating miRNAs as potential biomarkers.

MiR-126 is one of the most abundant miRNAs in ECs and plays a crucial role in regulating the function of ECs [52]. The transcription of miR-126 is regulated by several transcriptional factors, such as Krueppel-like factor 2 (KLF2) [52]. MiR-126 deficiency causes leaky vessels and hemorrhage because of loss of vascular in vivo, partly mediated by direct targeting of negative regulators of vascular endothelial growth factor (VEGF) signaling [53]. In one of the studies was determined that circulating miR-126 can be used as a potential diagnostic biomarker for IA and a predictive biomarker for judging IA rupture by detecting the expression of miR-126 in the serum received from peripheral venous blood of 102 IA patients [35]. The multi-factor analysis using the Lagrangian Relaxation (LR) method showed that the lesion size and circulating miR-126 expression were independent risk factors for IA patients rupture. In addition, through enrichment results of GO and KEGG, they found that miR-126 target genes are mainly enriched in biological functions, such as nucleus, transcription, DNA-templated, transcription factor activity, sequence-specific DNA binding, protein binding, and phosphatidylinositol phosphorylation, etc. KEGG analysis found that miR-126 target genes were significantly enriched in 44 pathways, such as the MAPK signaling pathway. And it is worth noting that the MAPK signaling pathway is expressed in ECs, VSMCs, and can also regulate vascular signal transduction pathways [54].

Powers et al. collected CSF via ventriculostomy from 8 patients with aSAH daily from days 3–12 post-aSAH [36]. There were 8 samples of aSAH CSF at each of the time points. At the same time, the authors carried out hierarchical clustering revealed two large clusters of miRNAs: one that contained miRNAs known to be present in CSF and decreased abundance over time and a second that showed increased abundance over time. In the first cluster, the overall downward trend was typified by the abundance of miR-92a and let-7b. In the second cluster, the overall increase over time was typified by the abundance of miR-491. These results were confirmed with quantitative qRT-PCR. These circulating miRNAs may have pleiotropic effects on brain injury and the brain's response to injury.

Circulating miRNAs and vasospasm

The main causes of death of patients with rupture of IAs are the direct effect of the spilled blood or SAH, hydrocephalus, and late cerebral ischemia (LCI) arising from vasospasm [3]. If the first two reasons are handled more successfully due to the improvement of the microsurgical technique for aneurysm operations and methods of controlling hydrocephalus, then the leading place among the complications is vasospasm and associated LCI [7]. Since cerebral vasospasm is a transient complication, its timely detection and prompt intervention can prevent LCI. It is known that the mechanism of vasospasm

after aSAH is based on a significant decrease in the level of nitric oxide (NO). NO is critical for the regulation of normal vascular tone, as a mediator of vasodilation, and the enzyme nitric oxide synthase (NOS) synthesizes NO. The causative role of NO in its occurrence is evidenced by the disappearance of NO synthase in vascular adventitia during a spasm, as well as the destruction of NO by hemoglobin, which is released from blood clots in the subarachnoid space [55]. Li et al. revealed a negative correlation between the expression levels of miR-24 and NOS3 mRNA in vascular tissue samples of patients with aSAH ($n = 29$), confirming the negative regulatory relationship between miR-24 and NOS3 [56]. In the present study, transfection with a miR-24 inhibitor increased the expression levels of NOS3, whereas transfection with a miR-24 mimic or NOS3 siRNA decreased NOS3 expression levels in vitro. In addition, miR-24 expression levels were increased in aSAH patients with vasospasm ($n = 13$) compared with those without ($n = 16$), whereas the opposite results were observed for NOS3. ASAH may cause changes in miR-24 expression in the cerebral arteries, which, once released into the bloodstream, can act as new biomarkers for assessing the risk of cerebral vasospasm and/or LCI, but all this requires additional studies.

Using NGS, Pulcrano-Nicolas et al. performed profiling circulating miRNAs in venous whole blood between vasospasm patients with aSAH and patients who did not develop vasospasm in a prospective cohort of 32 patients [57]. This study revealed that increased miR-3177-3p levels were associated with vasospasm risk in patients with aSAH. They also observed that this increase in miR-3177-3p levels was accompanied by a decrease in lactate dehydrogenase-A (LDHA) gene expression. LDHA expression in cerebral vascular endothelial cells was demonstrated to be influenced by hypoxia, where hypoxia is a key regulatory mechanism involved in vasospasm [37].

Su et al. compared the circulating miRNAs profiles of aSAH patients and healthy individuals ($n = 20$), and the circulating miRNAs profiles of aSAH patients with ($n = 20$) and without LCI ($n = 20$) [38]. In plasma isolated from peripheral venous blood of aSAH patients with LCI, a microarray study indicated that 99 circulating miRNAs were significantly dysregulated. Eighty-one circulating miRNAs were upregulated and 18 were downregulated. However, miR-132-3p and miR-324-3p showed distinctive upregulation in plasma of patients with LCI and in Non-LCI patients. From the ROC curve of miR-324, AUC was 0.97 for LCI patients and 0.96 for Non- LCI patients. MiR-132 gave an AUC of 0.75 for LCI patients and 0.73 for Non- LCI patients. The authors suggested that circulating miR-324-3p and miR-132 might be potential predictive biomarkers for rupture IAs and prognostic for LCI. Kikkawa et al. showed the time course of changes in the expression of circulating miR-15a and Krueppel-like factor 4 (KLF4) in the CSF and plasma (peripheral venous blood) following 8 patients with aSAH [39]. The expression level of miR-15a was increased in the CSF and plasma with a peak on days 3 and 5, respectively. Intriguingly, in the present study, KLF4 was dramatically decreased prior to the peak of miR-15a elevation both in the plasma and in CSF. Their results suggest that an early and persistent decrease in KLF4 followed by an increase in circulating miR-15a may contribute to vascular proliferation or angiogenesis, resulting in cerebral vasospasm.

Microarray analysis, using the plasma samples isolated from peripheral venous blood on three different stages (after the onset of SAH, the peak period of vasospasm and vasospasm dissipation phase) of patients with aSAH (20 patients with Hunt-Hess I-III), indicated that 2549 circulating miRNAs detectable in the plasma of the 9 sample pools [40]. In addition, according to the pathological development of aSAH, was selected three candidate circulating miRNAs (miR-3195, miR-4788, and miR-1914 by using the following filter conditions: (1) increase in the peak period of vasospasm compared with aSAH patients after the onset; and (2) decrease in vasospasm dissipation phase compared with the peak period of vasospasm. Furthermore, these circulating miRNAs were confirmed by qRT-PCR, where the expression levels of miR-3195, miR-4788, and miR-1914 were significantly higher in Phase II while could be decreased in Phase III of aSAH. Combined AUC in Phase I and Phase II was 0.976, while from Phase II to Phase III, the Combined AUC was 0.902, respectively. These data provide evidence that circulating miR-3195, miR-4788, and miR-1914 have the potential to be sensitive, cost-effective biomarkers for the dynamic monitoring of the development of aSAH.

In another study, Lopes et al. collected 33 peripheral whole blood samples (14 patients with vasospasm after aSAH, 13 patients without vasospasm after aSAH, and 6 healthy controls) [41]. Then, were performed NGS platform using a global circulating miRNAs expression analysis profile. Using NGS and qRT-PCR 8 miRNAs was found differentially expressed in aSAH patients, 3 being upregulated (miR-146a-5p, miR-589-5p, and hsa-miR-941) and 5 being downregulated (let-7f-5p, miR-126-5p, miR-17-5p, miR-451a, and miR-486-5p). In addition, circulating miR-486-5p was the most abundantly expressed and is associated with poor neurological admission status. These novel circulating miRNAs were predicted to be expressed only in aSAH, suggesting possible involvement in IA pathogenesis. For example, miR-146a-5p is involved in the regulation of inflammation and the regulation of innate immune responses in monocytes and macrophages. Also, Bache et al. reported a relative increase in circulating miR-146a-5p, miR-21, and miR-221 expression in CSF from 27 aSAH patients with LCI, compared with those without LCI. These findings may help the identification of novel biomarkers of clinical interest [42].

In the CSF of patients after aSAH (10 samples with vasospasm and 9 samples without vasospasm) and 4 control

patients, a microarray study indicated that 256 circulating miRNAs were differentially expressed [43]. CSF was collected daily: for the patients, the period ranged from 1 to 18 days. Among these miRNAs, 36 miRNAs exhibited fold change with a significant difference and undertook further examination to determine their target genes. Eighteen miRNAs of significantly differentially expressed were located within a single comparison group. They included miR-301a-3p, miR-378d, miR-137, miR-320e, miR-346, miR-514-3 pm, miR-521, miR-624-3p, miR-708-5p, miR-1244, miR-2117, miR-4521, miR-302a-3p, miR-548I, miR-566, miR-27a-3p, miR-516a-5p, and miR-1197. However, miR-451a showed significantly increased fold changes in a number of group comparisons. The authors observed lower expression levels of circulating miR-451a in patients who experienced aSAH with vasospasm (Day 1 samples) to patients who experienced aSAH without vasospasm. However, circulating miR-451a levels were also observed to increase in CSF samples analyzed post-Day 1 (Days 4–7 post-aSAH) from patients who experienced aSAH with vasospasm, which may counteract the early onset of a vasospastic phenotype linked to the phosphorylation of AMPK pathway components [58].

We believe that future studies with miRNAs should focus on the main and preventable cause of death and complications in patients with aSAH: cerebral vasospasm and/or LCI. Because, at present, the actual relationship between miRNA and cerebral vasospasm and/or LCI is still completely unknown, given that some patients suffering from cerebral vasospasm develop LCI, while in other patients with vasospasm - no. Therefore, the identification of new miRNAs and their targets will allow us to understand this mechanism after aSAH and to develop new biomarkers for circulating miRNAs for timely therapeutic intervention.

Exosomal miRNAs and IAs

Exosomes are small membrane vesicles of endosomal origin with a diameter of 30 to 100 nm. Exosomes participate in intercellular interaction, delivering their contents, like miRNAs, to target cells by direct contact between or without cells, and thereby affecting physiological and pathological processes [59, 60]. There are a number of studies proving the direct role of exosomal miRNAs in the development and progression of IAs [44, 45, 61]. Moreover, exosomal miRNAs can become excellent biomarkers for IAs. Feng et al. investigated the possible role of tumor-associated macrophage (TAM)-derived exosomes carrying miR-155-5p in the formation of IAs [60]. In their study was that miR-155-5p was highly expressed in TAM-derived exosomes. They also concluded miR-155-5p might be involved in the regulation of the proliferation and migration of VSMCs. Exosomes derived from TAMs transfected with miR-155-5p inhibitor have suppressed proliferation and migration of VSMCs in vitro. In a previous study, VSMCs-derived exosomes mediate the transfer miR-155 from VSMCs to ECs, which, in turn, destroys the integrity of endothelial barriers, leading to increased endothelial permeability and enhanced atherosclerotic progression [40]. Therefore, exosomal miR-155 plays an important role in the pathogenesis of IAs. Therefore, future studies with exosomal miR-155 should be aimed at finding new biomarkers for early diagnosis of IAs.

NGS analysis, using the plasma of patients with IAs, revealed that 181 miRNAs were identified to be differently [44]. Where 9 and 20 miRNAs were up-regulated, whereas 20 and 10 miRNAs were down-regulated in 69 patients with unruptured IAs ($n = 30$) and ruptured IAs ($n = 39$) compared with 30 healthy controls. In addition, 92 miRNAs were up-regulated, and 29 were down-regulated in patients with unruptured IAs compared with ruptured IAs. Furthermore, 2 circulating exosomal miRNAs (miR-145-5p and miR-29a-3p) were confirmed by qRT-PCR with significantly up-regulated in patient's plasma (peripheral venous blood) with unruptured and ruptured IAs. Most importantly, these miRNAs were of exosomal origin. This study investigated the exosome-derived miRNAs profiles in plasma from patients with IAs. ROC curve analysis showed that the AUC of miR-145-5p and miR-29a-3p was 0.737 and 0.791, respectively. In a previous study, demonstrated that miR-29a may contribute to the progression of IAs by regulating the mitochondrial apoptotic pathways [13]. Circulating exosomal miR-145-5p and miR-29a-3p profiles can serve as biomarkers for diagnosis and prognosis IAs. The next study was to evaluate the relationship of brain microvascular endothelial cells (BMECs) function and the exosomal miR-630 expression after aSAH [45]. The authors demonstrated that the expression of miR-630 was markedly reduced in the arterial blood-cerebrospinal fluid (BCSF) treated BMECs and the same phenomenon occurred in CSF of aSAH patient compared with normal hydrocephalus as control patients. In addition, the expression of ICAM-1, VCAM-1, and ZO-1 was then increased in BMECs co-cultured with exosomes transfected by miR-630 mimics. The decreases of ZO-1, ICAM-1, and VCAM-1 in BMECs reflect the ECs barrier protection dysfunction. This indicated a significant role of exosomal miR-630 in activating tight junction between cerebral vascular ECs which is the structural and functional base of the blood–brain barrier (BBB). Exosomal circulating miR-630 in CSF can certainly be used to study the pathological changes of cerebral microcirculation and be proposed as a potential biomarker for prognosis aSAH.

Another study demonstrates the potential of 25 circulating miRNAs derived from exosomes as biomarkers for differentiating between stroke subtypes including aSAH [46]. In this study was collected plasma samples from subjects suffering from aSAH ($n = 17$), intracerebral hemorrhage (ICH) ($n = 19$), and ischemic stroke (IS) ($n = 21$). LASSO analyses predicted a 24 circulating miRNAs classifier with an AUC of 0.977 and

an accuracy of 0.944 and 21 circulating miRNAs classifier with an AUC of 0.895 and an accuracy of 0.968 for discriminating aSAH from ICH and IS, respectively. In the 25 circulating miRNAs with the largest fold change, aSAH tended to be the most divergent subgroup. Among the 25 circulating miRNAs tested for aSAH, 20 upregulated and 5 downregulated. The utility of these circulating miRNAs as biomarkers to differentiate between stroke subtypes suggests that they could be developed into a point-of-care (POC) test that can be administered in the clinical to assist with diagnosis and patient triage. Further clinical testing of these circulating miRNAs panels can enhance the care of patients suffering from a stroke.

Lai et al. used NGS to define the global exosomal miRNAs profile in plasma of 3 aSAH patients and 3 healthy controls [47]. NGS permitted identification of a group of exosomal miRNAs that were differentially expressed in aSAH patients, yielding 6 circulating miRNAs that were significantly differentially expressed in aSAH patients compared to healthy controls: miR-369-3p, miR-136b-3p, miR-410-3p, miR-195-5p, miR-486-3p, and miR-193b-3p. In addition, the authors argue that confirmed these results through qRT-PCR of plasma samples from 10 patients with aSAH and 10 healthy controls. qRT-PCR confirmed that 4 exosomal miRNAs (miR-369-3p, miR-410-3p, miR-193b-3p, and miR-486-3p) showed significantly differential expression between the experimental group (24 h post-aSAH) and healthy controls. To further confirm the significance of the observed differences in exosomal miRNAs expression, the expression of exosomal miR-193b-3p in plasma remained statistically significant relative to controls in a mice aSAH model, whereas expression levels of miR-193b-3p were lower than controls in brain tissues of aSAH mice. Besides the authors suggested that RVG-exosomes containing miR-193b-3p may weaken neuroinflammation by suppressing the expression and the activity of HDAC3 and increasing NF-κB p65 acetylation after aSAH in vitro. Further studies are required to account for the benefits of exosomal miR-193b-3p as a biomarker.

Circulating miRNAs have emerged as attractive biomarkers since they are obtained through a non-invasive manner and have been reported to serve as diagnostic and prognostic tools for many human diseases, including IAs. However, many circulating miRNAs are passively released from apoptotic and necrotic cells and therefore may not precisely reflect the biological changes that occur in IAs. In contrast, different cell types, including ECs and VSMCs, actively secrete exosomes into the blood or CSF and they play a role in cerebrovascular protection and repair through miRNAs transfer [62]. Exosomes are a natural nanocarrier and intercellular messenger that plays a key role in the regulation of intercellular communication. Active interactions between EC and VSMC are critical to modulate the process of IA formation. This indicates that exosomes carry specific miRNAs as well as their miRNAs biogenesis machinery. Therefore, circulating exosomal miRNAs may truly represent specific molecular biomarkers compared with other circulating miRNAs. Indeed, several companies have initiated the development of exosome-based diagnostics. For example, a sensitive diagnostic of prostate cancer based on analysis of exosomal proteins was recently launched (http://www.CarisLifeSciences.com). It makes sense to develop molecular diagnostic tests based on exosomal miRNAs for use in personalized medicine. The main focus thus far is on oncology or diagnostic of the nervous system through exosome-based technology platforms (http://www.ExosomeDx.com; http://www.HansaBioMed.eu). Given that populations of exosomes in biological fluids are heterogeneous and can originate from all types of cells, especially blood cells, future research should determine the origin of exosomes present in biological fluids and whether exosomal miRNA levels correlate with specific cellular components of IA.

Future perspective and limitations

According to the working group of the National Institutes of Health Director's Initiative on Biomarkers and Surrogate Endpoints, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [63]. An ideal biomarker has high specificity and sensitivity, is detectable using minimally invasive procedures, and its concentration should indicate a disease state. Circulating miRNAs have a chance to be used both in diagnostics to assess the status of the disease, predictive information for the outcome of the disease, as well as to help determine the effectiveness of the treatment when comparing experimental groups with control ones. In recent years, NGS technologies have made it possible to simultaneously screen thousands of potential biomarkers in the form of circulating miRNAs, which contributes to both the detection of biosignatures of the expression of specific diseases, including IAs. Circulating miRNAs can also help in assessing the risk of rupture of AI, as well as provide information on the molecular mechanisms underlying LCI arising from vasospasm.

Although the market for miRNAs biomarkers is still in its infancy, some products are currently available to clinicians, while others are undergoing preclinical and clinical trials. One notable example is TAmiRNA: Circulating platelet miRNAs (thrombomiRs®) are novel biomarkers for intrinsic and on-treatment platelet reactivity. ThrombomiRs® are secreted from platelets during their activation and can be detected in plasma and serum.). Such case studies can provide invaluable information on the pathway of miRNA biomarkers from discovery to clinical application [64].

The sample type and route of the collection will impact the ease of use and clinical uptake of circulating miRNAs. Less

invasive samples, such as blood is often preferred over CSF. Indeed, circulating miRNAs have been found in almost all biofluids, however, the use of these less invasive sample types would depend on the underlying disease. For example, circulating miRNAs in CSF may be useful for the diagnosis of CNS tumors but may not be useful for tumors in other areas of the body [65]. Whole blood, serum, and plasma are often used for biomarker identification as peripheral samples often contain markers of localized disease. Consideration for disease pathology and clinical practice must be taken when looking to translate circulating miRNAs as biomarkers.

Lack of acceptable housekeeping gene(s) for data normalization is also another major limitation. Numerous endogenous miRNAs (e.g., hsa-miR-16) and small nuclear RNAs, such as RNU6B and RNU48, seem to be the most useful endogenous control for circulating miRNAs [66]. However, the expression of some of the endogenous miRNAs can change depending on the pathology, and the stability of RNU6B and RNU48 in biological fluids is still in doubt [67, 68]. The quantification of circulating miRNA can be strongly influenced by miRNA origin (for example, exosomes), and this can be a source of conflicting results. It should be noted that circulating miRNAs in plasma or serum can also be contaminated with miRNAs from other blood cells and require careful interpretation. Since a biomarker for SAH should discriminate between hemorrhage caused by ruptured IA or non-IA, the use of animal models is necessary for further study of tissue specificity of miRNA.

Finally, well-designed and large-scale prospective studies are required to validate the diagnostic utility of circulating miRNAs in early diagnosis of IA or prognosis aSAH. The studies should evaluate the effect of confounders and other conventional biomarkers on the diagnostic and prognostic models to improve the risk stratification rupture IA. Replication of findings in independent cohorts supported by experimental animal findings would minimize false-positive reports and highlight the plausible role of miRNA of interest. More importantly, efforts to better understand the biological processes controlling miRNA release and stability are needed. Although the correlation between circulating and tissue miRNA is still not clear, increasingly more data do not support the hypothesis that circulating miRNA levels reflect the specific changes that occur in the IA tissue, as miRNAs could also be derived from other cells like immune cells. However, from the data presented in this review, two particularly important conclusions should be drawn. First, the expression of circulating miRNAs was significantly altered compared to healthy controls in patients with ruptured and unruptured IAs. This finding supports the putative utility of circulating miRNAs as potential biomarkers for identifying IAs. Second, differential expression of circulating miRNA levels in biofluids in patients with IAs with and without daughter blebs may indicate changes in miRNA profiles at different time points in the development and progression of the IA. Thus, the cellular and molecular processes underlying the initiation, growth, and rupture of an IA can occur in different phases. Further understanding of the circulating miRNA profiles of these phases is a means by which one can better distinguish those aneurysms that are unlikely to rupture from these unstable IA with pronounced dome weakness. While fully validated miRNA-based diagnostic assays are available for diseases such as tumors future research studies are needed to establish the role of circulating miRNAs as diagnostic, predictive, and prognostic biomarkers in IAs and aSAH [69].

Table 2 summarized diagnostic, predictive, and prognostic factors of the individual circulating miRNAs, combined circulating miRNAs panels for patients with unruptured and ruptured IAs.

Conclusions

Circulating miRNAs hold great promise as diagnostic, prognostic, or predictive biomarkers in the clinical management of patients with IAs and aSAH. There is a fast-growing list of the original reports aimed to address the clinical value of circulating miRNAs in the diagnosis and prognosis of IAs. The number of these studies is expected to grow. This indicates a great interest and substantial potential benefits of circulating miRNAs in clinical medicine, but it also illustrates an existence of insufficient knowledge and a lack of conclusive information to clarify the role of circulating miRNAs in the IAs diagnosis and prognosis process.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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